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17 Contribution of the fungal population to the quality of dry-cured ham

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Abstract

The peculiar ecological characteristics of dry-cured ham, particularly the low a_w at the surface, are so favorable to fungi that the microbial population becomes largely dominated by yeasts and molds after the initial stages of processing. A diversity of yeast species has been found, being Debaryomyces hansenii the most common species reported in the different types of dry-cured hams. Penicillium spp. dominate the mold population when a_w is higher than 0.90, but Aspergillus and Eurotium dominate when a_w decreases. However, several Penicillium species, such as P. commune, P. expansun, P. chrysogenum can be isolated at the end of the ripening process. Most naturally occurring mold species are potentially mycotoxigenic. Therefore it is essential to control the fungal population growing on dry-cured ham. The use of non-toxigenic molds as starter cultures would

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effectively prevent unwanted fungal growth and production of secondary metabolites, keeping the positive contribution of the mold population. However, the contribution of fungi to the ripening of dry-cured ham is not fully understood. Most fungi isolated from dry-cured ham showed proteolytic activity for myofibrillar proteins, particularly P. chrysogenum, increasing the concentration of most free amino acids. The proteolytic effect of fungi also influences the generation of volatile compounds. Some compounds derived from auto-oxidation, such as linear aldehydes, ketones and aliphactic alcohols, have been reported at lower values when pork samples were inoculated with P. chrysogenum. Compounds derived from amino acids catabolism, such as branched aldehydes, branched carboxylic acids, and pyrazines showed higher values in pork inoculated with P. chrysogenum. The possible contribution of D. hansenii is not that clear. It does not seem to exert a decisive influence on formation of any of these compounds, except for some alcohols, and ketones. The results obtained with dry-cured hams inoculated with these two organisms confirmed the restriction of lipid auto-oxidation, but not the higher production of volatile compounds derived from amino acids. In addition, applying purified enzymes from selected molds or using yeasts expressing such enzymes would overcome the restrictions inherent to molds. Even though there is still some work to do, the selection of fungal starter cultures for dry-cured ham is of great interest and the main aspects are discussed here.

Introduction

Dry-cured ham is a traditional food in several countries, with a rather limited production when compared to other meat products. There is a number of different local varieties, ranging from "country style" hams ripened under uncontrolled environmental conditions for more than one year, to deboned "raw ham" manufactured under controlled conditions in just a few weeks. Most of these types of dry-cured hams are elaborated following traditional patterns that were developed empirically [1]. The usual number of ingredients is extremely short, and the different varieties share the essential aspects of processing. All this has made that research did not pay much attention to this product, which results in insufficient scientific knowledge of its processing.

In addition, the peculiar ecological characteristics of the product, particularly the low a_w at the surface, are so favorable to fungi that the microbial population becomes largely dominated by yeasts and molds after the initial stages of processing. The role of some of these in other fermented foods is well established. However, the contribution of these micro-organisms to the ripening of dry-cured ham is not fully understood.

Top quality products are highly appreciated by consumers, especially in Mediterranean countries. The high price they reach is encouraging studies to shorten ripening time and improve quality. This target requires understanding the role that micro-organisms and their enzymes play in this product.

As dry-cured ham is often made from entire pork legs, including bones and skin, it offers a quite singular environment for micro-organisms and an interesting system for food scientists.

Most fungi grow on the surface, where they outgrow other microbial groups becoming visible to the naked eye. Many molds have proved to be mycotoxigenic when grown on appropriate substrates. Even though many of such micro-organisms require rather stringent conditions to produce the toxins, their presence in meat products must be

considered a hazard as they may find the appropriate conditions in other foodstuffs. Total exclusion of molds from these products might solve the problem, but this would be really complex to achieve for products so favorable to mold growth and such a long ripening time. Nevertheless, some fungi are safe and could be used to control unwanted molds by competitive exclusion. For this, a deep insight in the ecology of this meat product of intermediate a_w is required.

On the other hand, the presence of fungi at high numbers for long ripening times would be enough to exert a strong influence on the characteristics of most foods, but not for dry-cured ham. A high proportion of the outer surface of this product is covered by skin and fat, limiting the proportion of muscle tissue exposed to micro-organisms. The tissue structure of these meat pieces, particularly the different layers of connective tissue and intramuscular fat, will block access of micro-organisms to deep tissues. Also, migration of enzymes has to be drastically limited during ripening. For this, the expected role of micro-organisms has been restricted merely to the muscle surface exposed to fungi. All this has made that the role of micro-organisms in dry-cured ham had been disregarded. In fact, the potential of endogenous muscle enzymes to ripening has been demonstrated, as it is shown in previous chapters of this book. However, the high numbers of the fungal population on the surface, the long ripening time of some country style hams, and the high activity of fungal enzymes may account for some contribution to aging. In addition, small soluble compounds formed at the surface may diffuse throughout the meat piece, thus contributing to changes in deep tissues.

The possible contribution of the fungal population to raw cured meat products was devised several years ago [1]. This would include antioxidant effect, creation of a favorable "micro-climate" on the surface, and breakdown of fats, proteins and lactic acid. Some molds have proved to be efficient producers of proteolytic enzymes. Of course, those growing on dry-cured ham should be able to obtain all their nitrogen and energy requirements from the substrate. This can be accomplished by diffusion of soluble compounds from deep tissues and by protein and lipid breakdown. Either of these should trigger changes in complex or simple compounds of the substrate. Such changes may not be considered deleterious, as they have been taking place in these products for ages with no correlation to undesired consequences. Quite opposite, they may exert a decisive influence on the sensorial characteristics of the final product. The possible contribution of fungi to dry-cured ham ripening is discussed in this chapter.

Fungal population on dry-cured ham

Since fungi are ubiquitous, they reach the ham surface from the slaughter and throughout processing. The environmental conditions of dry-cured ham ripening, mainly temperature and relative humidity, together with the physico-chemical characteristics, such as a_w and pH, select a population dominated by Gram-positive catalase-positive cocci, yeasts, and molds.

Fungi are found throughout the whole processing of dry-cured ham. Their counts reach 6-7 log c.f.u./g after salt equalization and first months of dry-curing stage [2,3], as temperatures increase to the maximum values of processing [4,5]. In the last months of ripening, counts decrease to 4-5 log c.f.u./g [3,6,7,8,9,10].

Yeasts

Yeasts are present in low numbers in fresh meat, but they outgrow bacteria when aw values limit bacterial metabolism. Thus, the yeast population reaches the highest counts on dry-cured hams during postsalting stage and firsts months of drying curing, when a_w falls below 0.95.

A diversity of yeast species has been found on dry-cured hams (Table 1). The population at early stages, included *Rhodotorula rubra* [2,11], *Hansenula sydowiorum, Hansenula holstii, Hansenula ciferrii, Rhodotorula glutinis, Cryptococcus albidus* [12], *Debaryomyces kloeckeri, Candida versatilis, Cryptococcus albidus, Saccharomyces lipolytica* [11], *Candida blankii, Candida intermedia, Candida zeylanoides, Debaryomyces hansenii* and *Pichia carsonii* [2]. Then, the diversity of species decrease with drying-curing time. Some yeasts are found when processing is shorter than one year and a_w is above 0.85. Among these, *C. zeylanoides, Geotricum candidum, Cryptococcus* spp., *Hansenula* spp., *Torulopsis* spp., and *Trichosporon* spp. have been reported [2,12,13]. *Debaryomyces* is the main yeast isolated from fully ripened hams [2,10,11,13,14], with *D. hansenii* as the most commonly reported yeast for the different types of dry-cured hams. This can be explained by its high salt tolerance [15,16], which makes it suitable for salted products such as dry-cured ham.

Table 1. Yeasts isolated from dry-cured hams

Type of ham	Stage	Refs.
Iberian		
Candida blankii	PS*	2
Candida zeylanoides	G, PS, F	2
Candida intermedia	PS	2
Debaryomyces hansenii	PS, F	2
Debaryomyces marama	F	10
Pichia carsonii	PS	2
Rhodotorula rubra	G, PS	2
Parma		
Debaryomyces hansenii	F	14
Geotricum candidum	F	14
Torulopsis candida	F	14
Torulopsis ernobii	F	14
Torulopsis famata	F	14
Torulopsis haemulonii	F	14
Trichosporon candidum	F	14
Trichosporon cutaneum	F	14
Trichosporon pullulans	F	14
Trichosporon variabile	F	14
Spanish Serrano		
Candida versatilis	PS	11
Cryptococcus albidus	PS, F	12
Debaryomyces hansenii	F	11
Debaryomyces kloeckeri	F	11
Hansenula ciferrii	PS, F	12
Hansenula holstii	PS, F	12
Hansenula sydowiorum	PS, F	12
Rhodotorula glutinis	PS	12
Rhodotorula rubra	PS	11
Saccharomyces lipolytica	PS, F	11
Portuguese		
Cryptococcus humicolus	F	13
Cryptococcus laurenti	F	13
Debaryomyces hansenii	F	13
Debaryomyces polymorphus	F	13

Stages: G: Green, PS: End of postsalting, D-C: Dry-curing, F: Final product

However, a great diversity of phenotypes or biotypes has been detected within species such as *C. zeylanoides* and *D. hansenii* [2,13]. According to biochemical and physiological characterization, 52 different biotypes of *D. hansenii* and 12 of *C. zeylanoides* have been isolated in just one study of Iberian dry-cured ham [2]. Some of these biotypes were found to be related to processing plant, while others found at different plants increased their proportion with ripening time [2]. Thus, yeast biotyping may be useful for estimating the progress of maturation.

Molds

Similarly to yeasts, molds cannot outgrow bacteria on raw meat. In fact, no significant fungal growth takes place on hams before salting. After that, the usual a_w decrease is unable to impede mold

growth, and counts increase to reach the highest values during post-salting and early drying-curing stages [3]. As a consequence, an abundant and uncontrolled mold population can be observed on the surface of different types of dry-cured hams, including American Country-style [18,19,20,21], Italian Parma [22,23], Austrian [24], Spanish Serrano [8,9,25,26], and Iberian [3,10] hams.

A number of mold species has been isolated from different types of dry-cured hams (Table 2). In contrast to yeasts, the diversity of molds increase throughout the ripening process, being maximum at drying-curing stages [3,25].

Table 2. Molds isolated from dry-cured hams

Type of ham	Stage	Refs.
Iberian		
Penicillium aurantiogriseum	PS, D-C, F	3
Penicillium brevicompactum	PS, F	3, 10
Penicillium commune	PS, D-C, F	3, 10
Penicillium chrysogenum	PS, D-C, F	3
Penicillium echinulatum	PS, D-C, F	3
Penicillium expansum	PS, D-C, F	3, 10
Penicillium nalgiovense	D-C	3
Penicillium oxalicum	F	3
Penicillium polonicum	PS, D-C, F	3, 27
Penicillium restrictum	D-C	3
Penicillium rugulosum	D-C	3
Penicillium viridicatum	PS, F	3, 10
Paecilomyvces variotti	D-C	3
Aspergillus niger	F	3
Aspergillus sydowii	F	3
Aspergillus versicolor	G	3
Eurotium herbariorum	D-C , F	3
Eurotium repens	D-C, F	3, 10
Eurotium rubrum	F	3
Alternaria tenuis	D-C	3
Aurobasidium pullulans	F	3
Cladosporium herbarum	F	3
Curvularia lunata	D-C	3
Syncephalastrum racemosum	F	3
Trichoderma viride	F	10
Parma		
Penicillium aurantiogriseum	F	22
Penicillium brevicompactum	F	23
Penicillium chrysogenum	F	23
Penicillium citrinum	F	23
Penicillium frequentans	F	22
Penicillium jensenii	F	23
Penicillium verrucosum	F	23
Penicillium waksmanii	F	23
Penicillium puberulum	F	23
Eupenicillium crustaceum	F	23
Eurotium amstelodami	F	23
Eurotium repens	F	23
Cladosporium spp	F	23
Speck (Tyrolean)		
Penicillium bervicompactum	F	24
Penicillium canescens	F	24
Penicillium chrysogenum	F	24
Penicillium commune	F	24
Penicillium glabrum	F	24
Penicillium nalgiovense	F	24
Penicillium solitum	F	24
Penicillium verrucosum	F	24
Penicillium waksmanii	F	24
Eurotium rubrum	F	24

Type of ham	Stage	Refs.
Spanish Serrano		
Penicillium chrysogenum	PS, D-C	25
Penicillium citrinum	D-C	25
Penicillium corylophilum	D-C	25
Penicillium cyaneofulvum	D-C	25
Penicillium digitatum	D-C	25
Penicillium expansum	D-C	25
Penicillium granulatum	D-C	25
Penicillium purpurogenum	D-C	25
Penicillium steckii	D-C	25
Aspergillus amstelodami	D-C, F	25
Aspergillus chevalieri	D-C, F	25
Aspergillus flavus	D-C	25
Aspergillus fumigatus	D-C	25
Aspergillus halophilicus	D-C, F	25
Aspergillus niger	PS, D-C	25
Aspergillus ochraceus	D-C	25
Alternaria tenuis	D-C	25
Cladosporium herbarum	PS, D-C	25
Fusarium moniliforme	PS, D-C	25
Rhizopus nigricans	PS, D-C	25
American Country style		
Penicillium aurantiogriseum	F	17
Penicillium brevicompactum	F	21
Penicillium chrysogenum	F	21
Penicillium citrinum	F	17
Penicillium commune	F	17
Penicillium expansum	F	21
Penicillium glabrum	F	17
Penicillium javanicum	F	17
Penicillium roqueforti	F	17
Penicillium viridicatum	F	20
Aspergillus amstelodami	F	19, 21
Aspergillus aurantiobruneus	F	19
Aspergillus candidus	F	19
Aspergillus chevalieri	F	21
Aspergillus conicus	F	19
Aspergillus flavus	F	18, 19
Aspergillus fumigatus	F	19, 21
Aspergillus gracilis	F	19
Aspergillus niger	F	17
Aspergillus ochraceus	F	18, 19
Aspergillus penicilloides	F	19
Aspergillus pseudoglaucus	F	19
Aspergillus repens	F	19, 21
Aspergillus restrictus	F	19, 21
Aspergillus ruber	F	19, 21
Aspergillus sydowii	F	19, 21
Aspergillus tamarii	F	18
Aspergillus versicolor	F	19

Stages: G: Green, PS: End of postsalting, D-C: Dry-curing, F: Final product

The dominant mold depends on the ecological conditions at the surface of the product. At the beginning of processing, when a_w is higher than 0.90, penicillia is the prevailing group [3,25]. The main *Penicillium* species, such as *P. commune, P. expansum,* and *P. chrysogenum*, have also been isolated at the end of the ripening process [3,10]. *Penicillium* is the most abundant mold in Parma and country-style hams [19,22,24]. However, *Aspergillus* and *Eurotium* spp. become dominant in Spanish and Iberian hams during dry-curing stages, when surface a_w decreases [3,10,25,26].

Most of the spontaneous mold species that colonize dry-cured ham are potentially mycotoxigenic [3]. Chloroform extracts of strains isolated from dry-cured ham were very toxic to brine shrimp larvae and Vero cells [3]. Moreover, strains of *Penicillium polonicum* and *P. commune* isolated from dry-cured hams produce verrucosidin and cyclopiazonic acid respectively on a meat extract based medium at a wide range of temperature (12-30°C) and a_w (0.99-0.90) [27,28]. The production of mycotoxins after experimental inoculation of molds on dry-cured ham, such as citrinin [20], sterigmatocystin [29], and ochratoxins [30] has been demonstrated. Thus, molds on dry-cured ham could pose a hazard to human health due to potentially hazardous fungal metabolites.

Therefore, it is essential to control the fungal population growing on dry-cured ham. Total exclusion of molds, would be difficult to accomplish due to the ecological conditions of this product. In addition it could impede any positive contribution from the mold population. Then, the application of starter cultures of non-toxigenic molds would be the most satisfactory way to effectively prevent the growth and secondary metabolite production by unwanted molds but keeping their positive contribution.

Lipolytic activity

Lipolysis in dry-cured hams has been attributed mainly to endogenous enzymes, which remain active during the whole ripening process [31]. Molds and yeasts could also contribute to lipolysis on hams, as different species isolated from dry fermented sausages [32,33] and dry-cured ham [25,34] have proved to be lipolytic.

As it has been reviewed in the previous chapter, a limited contribution of lipolysis is considered essential in dry-cured ham, but direct products of lipolysis do not play a decisive role in flavor. On the other hand, lipolysis facilitates lipid auto-oxidation, which is not considered a reaction to promote in meat products. Unsaturated pork fat and aerobic ripening at room temperature for several months guarantee lipid auto-oxidation over the desired level. For this, it is not advisable to increase the lipolytic potential of the microbial population.

Proteolytic activity

Muscle aminopeptidases remain active in dry-cured ham even after 8 months of processing [35]. These enzymes could be responsible for the increases in NPN reported on hams in the first stages of processing. However, curing agents [36] and the free amino acid released [37] produce a strong inhibitory effect on the muscle aminopeptidases. On the other hand, the microbial population growing on the surface might show amimopeptidase activity not affected by the level of amino acids present. This activity could be the main responsible for the increase in NPN reported on dry-cured hams in

drying stages [4]. In addition, the concentration of free Val, Met, Ile, Leu, His, Phe, and Tyr found at the surface are higher than those at depth in dry-cured ham [5]. This can be related to the higher microbial counts obtained at the surface [2,3,38], and the long processing time, up to 24 months, of dry-cured ham.

Most of the dominant organisms during the ripening process of dry-cured ham have shown a high proteolytic activity for myosin in a liquid culture media made up from nutrient broth [39]. The different organisms tested showed individual variations in relation to proteolysis on muscle proteins [39,40]. When selected strains of different groups were inoculated on pork slices, even micro-organisms tested as control for low proteolytic activity reduced the area of most proteins in relation to sterile controls [39]. Myofibrillar proteins seem to be more sensitive to proteolysis by these micro-organisms. Tested strains of *Penicillium chrysogenum, Penicillium commune, Paecylomyces variotti,* and *Debaryomyces hansenii* led to extensive hydrolysis of myofibrillar proteins in sterile meat slices [39,41]. Changes in the sarcoplasmic fraction were less significant, with *Staphylococcus xylosus* originating deeper effects.

These results had been obtained in pork slices and in culture media with myosin, where access of enzymes to proteins was fairly unrestricted. However, the tissue structure of dry-cured ham, including the different layers of connective tissue and intramuscular fat, may limit access of the micro-organisms and their enzymes to deep tissues. Thus, the effects observed on pork slices might not be reproduced in meat pieces with a low surface/volume ratio, and confirmation on dry-cured meat products was needed. One of the main obstacles to clarify the role of microorganisms on the ripening process of dry-cured ham was the lack of a sterile control to study changes on meat products for long ripening times. This difficulty has been overcome using a meat model system based on sterile pork loins ripened under aseptic conditions following a pattern for dry-cured ham [42].

One strain each of *D. hansenii* (Dh345) and non toxigenic *P. chrysogenum* (Pg222) [3], selected for their ability to produce major changes in most myofibrillar proteins, were assayed in such system [42]. None of the micro-organisms tested showed significant differences to sterile controls for sarcoplasmic proteins, even after 106 days of ripening. However, myofibrillar proteins were extensively hydrolyzed, particularly by *P. chrysogenum*, as compared to sterile control loins (Fig. 1). H-meromyosin, tropomyosin, and actin were significantly reduced in the batch inoculated with *P. chrysogenum* [42], thus confirming the results in pork slices [39,41]. The α-actinin band showed no significant difference between inoculated loins and sterile controls (Fig. 1), but this protein remains almost unaltered throughout the ripening process in dry-cured ham [4].

The proteolytic activity of these micro-organisms yields peptides and free amino acids. However, the amino acid content of pork samples inoculated with microorganisms requires a thorough examination. First, most amino acids increase during incubation of sterile pork [42]. This can be explained by the activity of muscle aminopeptidases with good stability during processing of dry-cured meat products [43]. Next, the micro-organisms growing on meat use amino acids as nitrogen and carbon sources, or transform them to new products. For this, the concentrations of Asp, Arg, and Leu were even lower in pork slices inoculated with *S. xylosus* or *D. hansenii* than in sterile controls [39]. Finally, microbial exopeptidases may account for increases in free amino

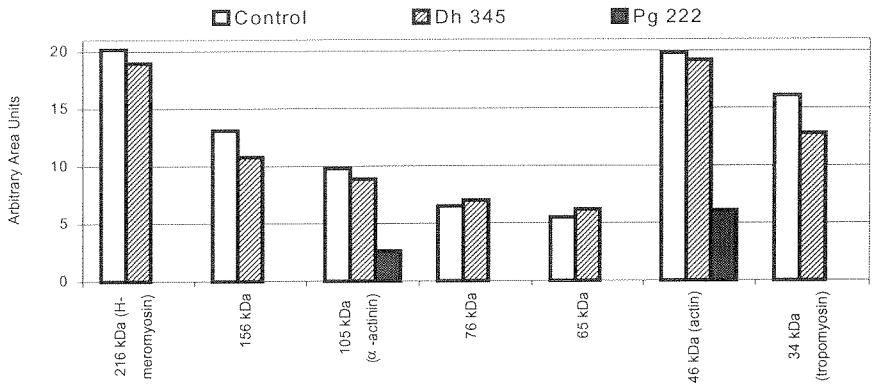

Figure 1. Myofibrillar proteins of loins inoculated with *P. chrysogenum* Pg222 and *D. hansenii* Dh345. Adapted from ref. 42.

acids, as it has been shown for semi-dry and dry-fermented meat products [44,45,46,47], particularly at the latter stages of proteolysis [48]. The selected fungal isolates of *P. chrysogenum, P. commune, Paecylomyces variotti*, and *D. hansenii* increased most free amino acids when grown on pork slices [39]. Some of these organisms proved also to be able to increase the concentration of several free amino acids in pork loins, where surface/volume ratio and tissue structure approximate to those of dry-cured ham. Samples inoculated with *P. chrysogenum* showed higher concentrations of Asp, Glu, Ser, Gly, Asn, Arg, Ala, Pro, Val, Met, Ile, Leu, Phe, and Lys than sterile controls [42] (Fig. 2).

Also yeasts, particularly *D. hansenii*, isolated from meat products increase some free amino acids in sausages [49]. However, this effect was not observed with three different strains of *D. hansenii* isolated form dry-cured ham when tested on pork slices [39] or pork loins [42] (Fig. 2).

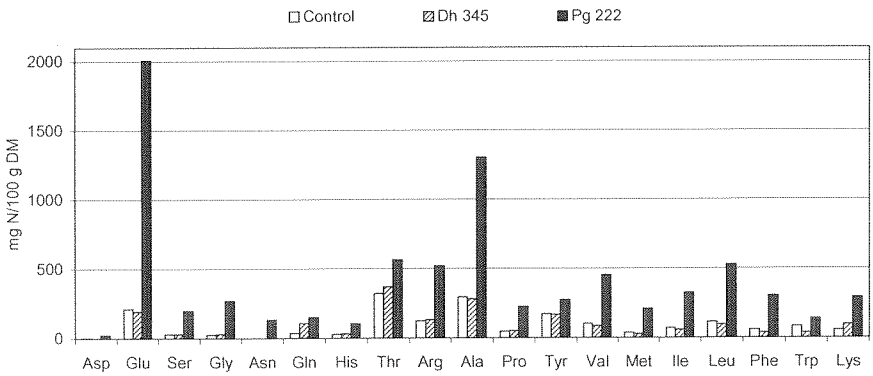

Figure 2. Free amino acids of pork loins inoculated with *P. chrysogenum* Pg222 and *D. hansenii* Dh345 and ripened in sterile conditions. Adapted from ref. 42.